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(54) TETRAPEPTIDES AND INTERMEDIATES THEREFOR

(71) We, E. R. SQUIBB & SONS INC., a Corporation organized under the laws of the State of Delaware, United States of America, of 745 Fifth Avenue, New York, United States of America, do hereby declare the inventon, for which we pray that a patent may be granted to us, and the method by which it is to be performed to be particularly described in and by the following statement:—

This invention relates to novel tetrapeptides and their chemically protected precursors. More particularly, this invention relates to novel therapeutically useful tetrapeptides of the general formula

R-L-prolyl-L-leucyl-L-glycinamide

and salts thereof, wherein R represents a radical selected from protected and unprotected radicals derived from an amino acid selected from glycine, tyrosin, leucine, triptophane, serine, 3-hydroxypicolinic acid, asparagine, phenylalanine, proline, glutamic acid, arginine and histidine.

Peptide salts encompased by the above formula include, for instance, hydrochlorides, hydrobromides, acetates, fluoroacetates, such as trifluoroacetate, chloroacetates, such as dichloroacetate, salts with amino acids and the like.

The novel compounds of this invention, wherein R, in the above formula, represents a free amino acid radical are active materials possessing antimicrobial properties inhibiting organisms such as *Staphylococcus, Salmonella, Pseudomonas, Proteus, Candida, Trycophyton, Trichophyton, Trichomonas, Escherchia,* and *Bacillus.* They are therefore useful as surface disinfectants in aqueous solutions or suspensions in concentrations of about 1 to 10% and also as laboratory reagents to prevent overgrowth of organisms such as the above when attempting to demonstrate the presence of other organisms such as Klebsiella species in cultures.

[*Price 25p*]

Compounds of this invention may also be employed in the control of estrus in farm animals, such as cattle. For this purpose, they may be administered usually in a single dosage, normally 1 to 50 mg./kg.

The compounds of this invention have further been found to be active immunosuppressive agents, inhibiting the immune antibody response in various animal species (e.g., mice). For this purpose, they may be administered in a dosage range generally of from 0.25 to 25 mg./kg. of body weight.

For these purposes, they may be administered orally or parenterally in such form as tablets, capsules or injectables, by incorporating the appropriate dosage of the compound with carriers according to standard pharmaceutical practices.

The products of this invention may be prepared beginning with the tripeptide L-prolyl-L-leucyl-L-glycinamide. The tripeptide is a known material, being the C-terminal sequence of the hormones oxytocin vasopressin. The selected amino acid is then added to this tripeptide to form the desired product.

Such addition is accomplished by first protecting the amino group of the amino acid to be added, as by forming its benzyloxycarbonyl derivative by methods well known in the art. The protected amino acid is then converted to one of its active forms, such as its nitrophenyl ester derivative, and interacting the thus protected, activated amino acid with the tripeptide to form the desired product.

The protecting groups which may be employed in the preparation of compounds of this invention are any of these protecting groups which are commonly employed in this art and include those exemplified below.

Among the suitable activating groups may be mentioned any group which causes the acid function to become more reactive, such

as a mixed anhydride, azide, acid chloride, reaction products with carbodiimides, and active esters, such as alkyl esters with electron attracting (negative) substituents, vinyl esters, enol esters, halo-phenyl esters, thiophenyl esters, nitrophenyl esters, 2,4-dinitrophenyl esters, and nitrophenylthiol esters. The use of nitrophenyl esters is particularly preferred from the standpoint of yield, lack of by-products, and consequent ease of purification.

In forming peptide sequences of this invention, the amino functions may be protected by commonly used amino protecting groups such as benzyloxycarbonyl, tertiary butyloxycarbonyl, phthalyl, *o*-nitrophenylsulfenyl and tosyl. Tertiary butyl, benzyl or nitrobenzyl, to give examples, may be used to protect the carboxyl group. The hydroxyl protecting groups may, for example, be benzyl, tertiary butyl, tetrahydropyranyl or acetyl, and the guanidine protecting groups may be nitro, tosyl or p-nitrobenzyloxycarbonyl, to give examples.

The protecting groups are removed by known reactions, such as reduction with sodium in liquid ammonia, hydrogenolysis (for instance, in the presence of a palladium on charcoal catalyst), treatment with a hydrohalo acid (such as hydrobromic or hydrochloric acids) in acetic acid or treatment with trifluoroacetic acid.

To prepare the free amines after treatment with a hydrohalo acid in acetic acid, the hydrobromide salt is treated either with an ion exchange resin such as "Amberlite IR400" or neutralized with an amine such as triethylamine. ("Amberlite" is a Registered Trade Mark.)

The following examples further illustrate the invention. All temperatures are in degrees centigrade unless otherwise stated.

Example 1

N-Benzyloxycarbonylglycyl-L-prolyl-L-leucylglycinamide

To a suspension of 8.8 g. (30 mmoles) L-prolyl-L-leucylglycinamide in 15 ml. dimethylformamide (DMF), while stirring, 11 g. (33 mm.) of benyloxycarbonyl glycine-p-nitrophenyl ester is added. The mixture is stirred at room temperature for about a half-hour until the solution is complete. The solution is allowed to stand overnight and then diluted with ethyl acetate (100 ml.) when the product separates out. The insoluble material is filtered off and washed with ethyl acetate and dried in vacuo, at room temperature, over phosphorous pentoxide. The crude preparation can be crystallized from 95% ethanol to give an analytically pure product melting at 192-195°, (yield 95%).

ANAL. Calc'd. for $C_{25}H_{33}O_6N_5$:
C, 58.09; H, 7.00; N, 14.73
Found: C, 57.92; H, 7.02; N, 14.52

Example 2

Glycyl-L-prolyl-L-leucylglycinamide hydrochloride

The above protected tetrapeptide 9.5 g. (20 mm.), is dissolved in 200 ml. 95% ethanol and hydrogenated at room temperature in the presence of one equivalent of normal hydrochloric acid and an atmospheric pressure and 1 g. of 5% palladium on charcoal until the test for the evolution of carbon dioxide is negative (circa 2 hours). The catalyst is then removed by filtration and washed with ethanol. The combined filtrate and washings are evaporated *in vacuo* and the residue triturated with ethanol and the solvent evaporated. This residue, when treated with chloroform, solidified. It is filtered off and washed with chloroform and then dissolved in boiling ethanol. On concentration to one-fifth of this solution, crystals begin to appear. On standing in the cold the product crystallizes out. It is filtered off, washed with alcohol and air-dried. The tetrapeptide when heated darkens at 230° and melts with decomposition at 233-235°, (yield 80%); $[\alpha]_D^{30}$-85° (C 1, 95% EtOH).

ANAL. Calc'd. for $C_{19}H_{32}N_6O_4 . HCl$:
C, 47.68; H, 7.47; N, 18.74; Cl, 9.38
Found: C, 47.64; H, 7.56; N, 18.69; Cl, 9.52

Example 3

O-Benzyl-N-benzyloxycarbonyl-L-tyrosyl-L-prolyl-L-leucylglycinamide

A mixture of 5.8 g. (20 mm.) of L-prolyl-L-leucyl-glycinamide in 25 ml. of DMF is treated with 11.2 g (20 mm.) O-benzyl-N-benzyloxycarbonyl-L-tyrosine-p-nitrophenyl ester as in Example 1 above. The product, which can be crystallized from 95% ethanol, melts at 173-175° (yield 95%).

ANAL. Calc'd. for $C_{37}H_{45}N_5O_7$:
C, 66.15; H, 6.75; N, 10.43
Found: C, 66.37; H, 6.90; N, 10.56

Example 4

L-Tyrosyl-L-prolyl-L-leucylglycinamide hydrochloride

The protected tetrapeptide obtained above is dissolved in glacial acetic acid containing one equivalent of normal hydrochloric acid and hydrogenated over a palladium/charcoal catalyst as in Example 2 for nineteen hours to remove the protecting groups. The product that crystallizes from ethanol/ethyl acetate mixture contains bound acetic acid and melts at 83-85°. Crystallization from ethanol (95%) by the addition of ether forms a crystalline hemihydrate salt which begins to soften at about 140° and melts with decomposition over a range 150-170°, (75% yield); $[\alpha]_D^{26}$-30° (C 1, EtOH).

ANAL. Calc'd. for $C_{32}H_{33}N_5O_5HCl.1/2H_2O$: C, 53.61; H, 7.16; N, 14.21; Cl, 7.19
Found: C, 53.85; H, 8.44; N, 14.17; Cl, 7.24

Example 5

N-Beizyloxycarbonyl-L-leucyl-L-prolyl-L-leucylglycinamide

A suspension of 5.8 g. (20 mm.) L-prolyl-L-leucyl-glycinamide in 10 ml. DMF is treated with 8.5 g. (22 mm.) benxyloxycarbonyl-L-leucine-p-nitrophenyl ester as in Example 1, and the product isolated from the reaction mixture following dilution of the reaction mixture with ether. It is purified by crystallization from benzene/ether mixture. It softens about 70° and melts at 85° (70% yield).
ANAL. Calc'd. for $C_{32}H_{41}N_5O_6$:
C, 60.99; H, 7.77; N, 13.17
Found: C, 60.23; H, 8.97; N, 13.42

Example 6

L-Leucyl-L-prolyl-L-leucylglycinamide hydrochloride

The above protected tetrapeptide is hydrogenated for about two hours in ethanol as described in Example 2 and the product is obtained crystalline from an alcohol-ether mixture. It is a hygroscopic material which softens at 150° and gradually decomposes up to 180°; 90% yield; $[\alpha]_D^{23}$-78° (C 1, 95% EtOH).
ANAL. Calc'd. for $C_{19}H_{35}N_5O_4 . HCl$:
C, 52.58; H, 8.36; N, 16.40; Cl, 8.17
Found: C, 52.27; H, 8.48; N, 16.09; Cl, 8.14

Example 7

N-Benzylcarboxycarbonyl-L-tryptophyl-L-prolyl-L-leucylglycinamide

To a suspension of L-prolyl-L-leucyl-glycinamide, 5.8 g. (20 mm.) in DMF, 9.3 g. benzyloxycarbonyl-L-tryptophane, p-nitrophenyl ester is added and allowed to react. The product is isolated by dilution with ethyl acetate and hexane. It is crystallized from 95% ethanol in the form of a monohydrate, melting at 143-145°C. (80% yield).
ANAL. Calc'd for $C_{32}H_{46}N_6O_6 . H_2O$:
C, 61.72; N, 6.80; N, 13.50
Found: C, 61.52; H, 7.25; N, 13.94

Example 8

L.Tryptophyl-L-prolyl-L-leucylglycinamide

The above tetrapeptide is hydrogenated at room temperature in 95% ethanol containing 5% actice acid. The free tetrapeptide is isolated from an alcohol solution after the addition of ether. It softens when heated at about 115° and melts gradually at 130-135° $[\alpha]_D^{26}$-31.5 (C 1, 95% EtOH).
ANAL. Calc'd. for $C_{21}H_{34}N_6O_4$:
N, 17.86.
Found: N, 17.71

Example 9

O-Actyl-N-benzyloxycarbonyl-L-seryl-L-prolyl-L-leucylglycinamide

A suspension of 20 millimoles (5.8 g.) of L-prolyl-L-leucylglycinamide in 10 ml. of DMF is allowed to react with 8.5 g. (21 mm.) of O-acetyl-N-benzyloxycarbonyl-L-serine, p-nitrophenyl ester as described in Example 1. The product isolated from the reaction mixture could be purified by crystallization from benzene or ethyl acetate. It melts at 147-150°. (70% yield).
ANAL. Calc'd. for $C_{25}H_{37}N_5O_8$:
C, 57.02; H, 6.81; N, 12.79
Found: C, 57.41; H, 7.36; N, 12.82

Example 10

L-Seryl-L-prolyl-L-leucylglycinamide hydrochloride

The above protected tetrapeptide is dissolved in ethanol and hydrogenated in the presence of 5% palladium on charcoal and one equivalent of hydrochloric acid. The free tetrapeptide salt is isolated from the reaction mixture as in Example 2 and crystallized from ethanol in 75% yield, m.p. 227-230° (decomp); $[\alpha]_D^{22}$-80.4 (C 1, 95% EtOH).
ANAL. Calc'd. for $C_{16}H_{31}N_5O_5 . HCl$:
C, 47.11; H, 7.41; Cl, 8.69
Found: C, 46.95; H, 7.87; Cl, 8.85

Example 11

3-Benzyloxypicolinyl-L-prolyl-L-leucylglycinamide

In a suspension of 5.8 g. (20 mm.), L-prolyl-L-leucylglycinamide in 10 ml. of DMF, 3-benzyloxypicolinic acid, p-nitrophenyl ester is allowed to react. The protected tetrapeptide formed is isolated from the reaction mixture by precipitation with ethyl acetate/hexane or ether. It is purified by crystallization from ethyl acetate, m.p. 154-155° (75% yield).
ANAL. Calc'd. for $C_{26}H_{33}N_5O_5$:
C, 63.01; H, 6.71; N, 14.13
Found: C, 62.87; H, 6.88; N, 14.24

Example 12

3-Hydroxypicolinyl-L-prolyl-L-leucylglycinamide

The above protected tetrapeptide is hydrogenolyzed in ethanol containing 10% acetic acid with a 5% palladium on charcoal catalyst. The free tetrapeptide obtained is crystallized from ethanol, m.p. 200-203° (65%); $[\alpha]_D^{22}$-95° (C 1, 95% EtOH).
ANAL. Calc'd. for $C_{19}H_{27}N_5O_5$:
C, 56.28; H, 6.71; N, 17.27
Found: C, 56.35; H, 6.86; N, 17.26

Example 13

N—Benzyloxycarbonyl-L-asparaginyl-L-prolyl-L-leucylglycinamide

Benzyloxycarbonyy-L-asparagine, p-nitrophenyl ester, 8.5 g. (21 mm.) and L-prolyl-L-leucylglycinamide, 5.8 g. (20 mm.) are allowed to react in 10 ml. of DMF as in Example 1 and the product isolated by precipitation with ether. It is crystallized from a mixture of alcohol/ether as the monohydrate, m.p. 188-189°; (86% yield).

ANAL. Calc'd. for $C_{27}H_{43}N_7O_7 . H_2O$:
C, 54.53; H, 6.96; N, 15.26
Found: C, 54.78; H, 6.59; N, 15.20

Example 14

L-Asparaginyl-L-prolyl-L-leucylglycinamide hydrochloride

The above protected tetrapeptide is hydrogenated in 80% acetic acid using a 5% palladium on charcoal catalyst. The product is treated with an alcohol solution of hydrogen chloride to form the hydrochloride salt which is precipitated by the addition of ether. It is crysallized from alcohol by careful dilution with ether. After drying in air, the compound forms a monohydrate which softens at about 105° and melts with decomposition by 150° (90%yield), $[\alpha]_D^{23}$-66° (C 1, 95% ethanol).

ANAL. Calc'd. for $C_{17}H_{30}N_6O_5 . HCl . H_2O$:
C, 45.08; H, 7.34; N, 18.55; Cl, 7.84
Found: C, 45.52; H, 7.25; N, 17.94; Cl, 8.23

Example 15

N-Benzyloxycarbonyl-L-phenylalanyl-L-prolyl-L-leucylglycinamide

A mixture of 5.8 g. (20 mm.) of L-prolyl-L-leucylglycinamide and 8.8 g. (21 mm.) benzyloxycarbonyl-L-phenylalanine, p-nitrophenyl ester are allowed to react in 10 ml. of DMF as outlined in Example 1 and the product isolated by precipitation with ethyl acetate. It is crystallized from ethyl acetate in nearly quantitative yield, m.p. 179-180°.

ANAL. Calc'd. for $C_{30}H_{39}N_5O_6$:
C, 63.70; H, 6.95; N, 12.38
Found: C, 63.87; H, 6.93; N, 12.20

Example 16

L-Phenylalanyl-L-prolyl-L-leucylglycinamide hydrochloride

The above protected tetrapeptide is dissolved in 95% ethanol containing one equivalent of hydrochloric acid and hydrogenated using a 5% palladium on charcoal catalyst. The isolated tetrapeptide salt is purified by dissolving in ethanol and precipitating with ether, m.p. 140-150° (d); $[\alpha]_D^{22}$-40° (C 1, 95% EtOH).

ANAL. Calc'd. for $C_{21}H_{33}N_5O_4 . HCl$:
C, 56.46; H, 7.32; N, 14.97; Cl, 7.58
Found: C, 56.26; H, 7.54; N, 14.59; Cl, 7.34

Example 17

Benzyloxycarbonyl-L-prolyl-L-prolyl-L-leucylglycinamide

A mixture of 5.8 g. (20 mm.) of L-prolyl-L-leucylglycinamide and 8.1 g. (21 mm.) of benzyloxycarbonyl-L-proline, p-nitrophenyl ester are allowed to react at room temperature in 10 ml. DMF. The product is isolated by dilution of the reaction mixture with ether. The product is crystallized from ethyl acetate, m.p. 123-125°, (95% yield).

ANAL. Calc'd. for $C_{32}H_{47}N_7O_8$:
C, 60.56; H, 7.23; N, 13.58
Found: C, 60.59; H, 7.35; N, 13.81

Example 18

L-Prolyl-L-prolyl-L-leucylglycinamide hydrochloride

The above protected tetrapeptide is dissolved in 95% ethanol containing one equivalent of hydrochloric acid and hydrogenated using a 5% palladium on charcoal catalyst. The product which solidifies on treatment with ether is purified by dissolving in methanol and precipitating with ethyl acetate. The product softens at 135° and melts with decomposition by 150° (90% yield) $[\alpha]_D^{22}$-132° (C 1, 95% ethanol).

ANAL. Calc'd. for C, 51.73; H, 7.72; N, 16.76; Cl. 8.48
Found: C, 51.63; H, 7.89; N, 16.53; Cl, 8.36

Example 19

N-Benzyloxycarbonyl-L-glutamyl(γ-t-butyl ester)-L-prolyl-L-leucylglycinamide

A suspension of 5.8 g. (20 mm.) of L-prolyl-L-benzylglycinamide in 10 ml. of DMF is allowed to react with 10 g. (22 mm.) of N-benzyloxycarbonyl-L-glutamic acid, α-p-nitrophenyl ester, γ-t-butyl ester as in Example 1. The product is isolated by diluting the reaction mixture with ether and is purified by crystallization from ethyl acetate, yield 80%, m.p. 158-159°.

ANAL. Calc'd. for $C_{30}H_{45}N_5O_8$:
C, 59.68; H, 7.51; N, 11.60
Found: C, 59.77; H, 7.59; N, 11.73

Example 20

L-Glutamyl-L-prolyl-L-leucylglycinamide

A solution of 7.5 g. (13 mm.) of the above protected tetrapeptide in 65 ml. of anhydrous trifluoroacetic acid is allowed to stand at room temperature for twenty minutes. The solvent is then evaporated *in vacuo* and the residue dissolved successively in toluene and benzene and the solvents removed *in vacuo*. The final residue is treated with ether and becomes solid. It softens about 70-75° and decomposes about 100°; nitrogen analysis indicates 12.88%; N-benzyloxycarbonyl-L-

glutamyl-L-prolyl-L-leucylglycinamide requires 12.79%. This intermediate is dissolved in 40% ethanol containing 1% acetic acid and is hydrogenated using a 5% palladium on charcoal catalyst. The product is obtained as a solid after treating with alcohol and ether (80% yield). It softens when heated at about 90° and melts with decomposition 130-135° (a 1% solution in ethanol on slow crystallization yielded a material with m.p. 158-159°); $[\alpha]_D^{23}$-66° (C 1, MeOH).

ANAL. Calc'd. for $C_{18}H_{31}N_7O_6 . H_2O$:
C, 50.10; H, 7.71; N, 16.23
Found: C, 49.48; H, 7.92; N, 16.02

Example 21
N-Benzyloxycarbonyl-(nitro)-L-arginyl-L-prolyl-L-leucylglycinamide

A suspension of 5.8 g. (20 mm.) of L-prolyl-L-leucylglycinamide in 10 ml. DMF is allowed to react with 16 g. (31 mm.) N-benzyloxycarbonyl-nitro-L-arginine, 2,4-dinitrophenyl ester as in Example 1. The product is isolated from the reaction mixture following dilution with ethyl acetate, and purified by dissolving in ethanol and precipitating with ether. The product softens on heating at 85° and melts with decomposition by 95° (70% yield).

ANAL. Calc'd. for $C_{27}H_{41}N_9O_8 . H_2O$:
C, 52.04; H, 6.79; N, 19.77
Found: C, 51.78; H, 6.51; N, 18.50

Example 22
L-Arginyl-L-prolyl-L-leucylglycinamide hydrochloride

A portion 7.6 g. (12 mm.) of the above protected tetrapeptide is dissolved in 80% acetic acid and hydrogenated using 5% palladium on charcoal as catalyst until the absorption of hydrogen ceases (48 hours). After the removal of the catalyst, one equivalent of N-hydrochloric acid is added to the mother liquor and the latter evaporated *in vacuo*. The residue is treated successively with ethanol and ethyl acetate until a hard morphous solid is obtained. This is purified by counter-current distribution in a series of 30-transfers using a solvent system of n-butanol-ethanol-water (4:1:5). The contents of the tubes (2-6) (containing the ninhydrin and Sakaguchi positive material) are combined and the resultant solution treated with triethylamine (3 ml.) and filtered. The solvent is removed *in vacuo* and the residue successively triturated with ethyl acetate and chloroform and dried over sodium hydroxide. The product melts with decomposition about 85-90° (yield 70%); $[\alpha]_D^{23}$-47° (C 1.3, 95% ethanol) and a quantitative amino acid analysis gives a ratio of arginine : proline : leucine : glycine of (0.99: 0.95: 0.99:1) for the tetrapeptide.

ANAL. Calc'd. for $C_{29}H_{50}N_8O_5 . HCl . H_2O$:
C, 46.10; H, 7.94; Cl, 7.16
Found: C, 46.37; H, 8.80; Cl, 6.92

Example 23
N-Benzyloxycarbonyl-L-histidyl-L-prolyl-L-leucylglycinamide

N-Benzyloxycarbonyl-L-histidine hydrazide, 6.6 g. (22 mm.) is converted to the corresponding azide; extracted into ethyl acetate and the extract added to a suspension of 5.8 g. (20 mm.) of L-prolyl-L-leucylglycinamide in 10 ml. DMF. The ethyl acetate is removed by evaporation *in vacuo* at room temperature and the reaction mixture allowed to stand overnight at 5°C. The product is isolated after the removal of DMF under vacuum distillation, by treating the residue successively with ethyl acetate and ether, when it solidifies. It is purified by extracting with boiling benzene (6 l.) and through crystallization from ethyl acetate by concentrating a solution of the product in this solvent up to the point of opalescence. The product melts at 150-153° (70% yield).

ANAL. Calc'd. for $C_{27}H_{37}N_7O_6$:
C, 58.36; H, 6.71; N, 17.65
Found: C, 58.34; H, 7.40; N, 17.38

Example 24
L-Histidyl-L-prolyl-L-leucylglycinamide dihydrobromide

The above protected tetrapeptide is dissolved in glacial acetic acid and an equal volume of 40% hydrogen bromide in acetic acid added. The reaction mixture is allowed to stand at room temperature for two hours and then diluted with ether to precipitate the product. The latter is purified by dissolving in a large volume of methanol, filtering and then concentrating to a small volume when the tetrapeptide salt separates in crystalline form (75% yield), m.p. 230-233° (d); $[\alpha]_D^{23}$-72.5° (C 1.1, 95% ethanol).

ANAL. Calc'd for $C_{19}H_{33}N_7O_4 . 2HBr$:
C, 39.12; H, 5.84; N, 16.80; Br, 27.40
Found: C, 38.94; H, 5.96; N, 17.05; Br, 27.14

WHAT WE CLAIM IS:—

1. A compound of the formula
R-L-prolyl-L-leucyl-L-glycinamide
or a salt thereof, wherein R is glycyl, N-protected glycyl, L-tyrosyl, N-protected-O-protected-L-tyrosyl, L-leucyl, N-protected-L-leucyl, L-triptophyl, N-protected-L-tryptophyl, L-seryl, N-protected-O-protected-L-seryl, L-asparaginyl, N-protected L-asparaginyl, L-phenylalanyl, N-protected L-phenylalanyl, L-prolyl, N-protected L-prolyl, L-glutamyl (γ-t-butyl ester), L-arginyl, N-protected nitro-L-arginyl, L-histidyl, N-protected-L-histidyl, 3-hydroxypicolinyl, or O-protected 3-hydroxypicolinyl.

2. A compound as claimed in Claim 1

wherein the amino protective groups are selected from benzyloxycarbonyl, tertiary butyloxycarbonyl, phthalyl, *o*-nitrophenylsulfenyl and tosyl, the carboxyl protective groups are selected from tertiary butyl, benzyl and nitro-benzyl, the hydroxyl protecting groups are selected from tertiary butyl, benzyl and tetrahydropyranyl, acetyl and the guanidine protecting groups are selected from nitro, tosyl and *p*-nitrobenzyloxycarbonyl.

3. N - benzyloxycarbonylglycyl - L - prolyl - L - leucylglycinamide.

4. Glycyl - L - prolyl - L - leucylglycinamide hydrochloride.

5. O - benzyl - N - benzyloxycarbonyl - L - tyrosyl - L - prolyl - L - leucylglycinamide.

6. L - tyrosyl - L - prolyl - L - leucylglycinamide hydrochloride.

7. N-benzyloxycarbonyl - L - leucyl - L - prolyl - L - leucylglycinamide.

8. L - leucyl - L - prolyl - L - leucylglycinamide hydrochloride.

9. N - benzyloxycarbonyl - L - tryptophyl - L - prolyl - L - leucylglycinamide.

10. L - tryptophyl - L - prolyl - L leucylglycinamide.

11. O - acetyl - N - benzyloxycarbonyl - L - seryl - L - prolyl - L - leucyl - glycinamide.

12. L - seryl - L - prolyl - L - leucylglycinamide hydrochloride.

13. 3 - benzyloxypicolinyl - L - prolyl - L - leucylglycinamide.

14. 3 - Hydroxypicolinyl - L - prolyl - L - leucylglycinamide.

15. N - benzyloxycarbonyl - L - asparaginyl - L - prolyl - L - leucylglycinamide.

16. L - asparaginyl - L - prolyl - L - leucylglycinamide hydrochloride.

17. N - benzyloxycarbonyl - L - phenylalanyl - L - prolyl - L - leucylglycinamide.

18. L - phenylalanyl - L - prolyl - L - leucylglycinamide hydrochloride.

19. Benzyloxycarbonyl - L - prolyl - L - prolyl - L - leucylglycinamide.

20. L - prolyl - L - prolyl - L - leucylglycinamide hydrochloride.

21. N - benzyloxycarbonyl - L - glutamyl (γ - t - butyl ester) - L - prolyl - L - leucylglycinamide.

22. L - glutamyl - L - prolyl - L - leucylglycinamide.

23. N - benzyloxycarbonyl - (nitro) - L - arginyl - L - prolyl - L - leucylglycinamide.

24. L - arginyl - L - prolyl - L - leucylglycinamide hydrochloride.

25. N - benzyloxycarbonyl - L - histidyl - L - prolyl - L - leucylglycinamide.

26. L - histidyl - L - prolyl - L - leucylglycinamide dihydrobromide.

27. A process for the preparation of a compound of the formula:

R-L-prolyl-L-luecyl-L-glycinamide

or a salt thereof, wherein R is as defined in claim 1, which comprises converting an amino acid, wherein the amino acid radical is as defined in R above and is in a protected form, to one of its active forms, and then interacting the thus protected activated amino acid with a tripeptide of the formula

L-prolyl-L-leucyl-L-glycinamide

and optionally removing the protecting group.

28. A process as claimed in claim 27, wherein the amino radical protecting group is a benzyloxycarbonyl derivative.

29. A process as claimed in claim 27, wherein the amino acid is converted into its active form as its nitrophenyl ester derivative.

30. A compound as claimed in claim 1, substantially as herein described.

31. A process as claimed in claim 27 for the preparation of a compound as therein defined, substantially as herein described.

32. A compound as claimed in any of claims 1 to 26 and 30 which has been prepared using a process as claimed in any of claims 27 to 29 and 31.

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